Orientation of colonized sand flies *Phlebotomus papatasi, P. duboscqi,* and *Lutzomyia longipalpis* (Diptera: Psychodidae) to diverse honeys using a 3-chamber in-line olfactometer

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ABSTRACT: A 3-chamber in-line olfactometer designed for use with sand flies is described and tested as a high-throughput method to screen honeys for attractiveness to *Phlebotomus papatasi* (four geographic isolates), *P. duboscqi* (two geographic isolates), and *Lutzomyia longipalpis* maintained in colonies at the Walter Reed Army Institute of Research. A diversity of unifloral honey odors were evaluated as a proxy for the natural floral odors that sand flies may use in orientation to floral sugar sources in the field. In the 3-chamber in-line olfactometer, the choice modules come directly off both sides of the release area instead of angling away as in the Y-tube olfactometer. Of the 25 honeys tested, five had a significant attraction for one or more of the sand fly isolates tested. This olfactometer and high-throughput method has utility for evaluating a diversity of natural materials with unknown complex odor blends that can then be down-selected for further evaluation in wind tunnels and/or field scenarios. ***Journal of Vector Ecology* 39 (1): 94-102. 2014.**

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INTRODUCTION

Phlebotomine sand flies are significant vectors that transmit several human and animal pathogens in warm tropical, semi-arid, and arid environments around the world. As the only vector of human leishmaniases they put at risk an estimated 350 million people with an annual incidence estimated at 1–1.5 million cases of cutaneous leishmaniasis, 500,000 cases of visceral leishmaniasis, and a Disability Adjusted Life Years of 2.1 million (Desjeux 2001, Hide et al. 2007, Antinori et al. 2012, Alvar et al. 2012). Leishmaniasis is a major cause of infectious disease morbidity among military personnel deployed to the Middle East and Afghanistan (Coleman et al. 2009). Leishmaniasis appears to be emerging globally, primarily due to anthropogenic effects (Ashford 2000, Desjeux 2001, Wasserberg et al. 2003, Harrus and Baneth 2005). Although therapeutic treatments are available, cost, access, and side effects limit their effectiveness. With no vaccine to protect against the etiologic agent, reduction of exposure to sand fly bites is the most effective prevention measure (Murray et al. 2005, Antinori et al. 2012).

Sand fly control comprises three general approaches: personal protection (e.g., repellents, insecticide-treated clothing, or bednets), reservoir host control (e.g., rodent removal using rodenticides or by burrow plowing), or residual spraying of insecticides (Alexander and Maroli 2003). The most common approach is the use of residual spray, but this method exhibits varying degrees of success and is more effective in urban than in rural areas (Ashford 1999, Alexander and Maroli 2003), has a potential to affect a wide range of non-target insects (Pimentel 1995), and in some cases produces insecticide resistance (Alexander et al. 2009, Dinesh et al. 2010, Hassan et al. 2012). Source reduction using larvicides is not practical for sand flies, which unlike most biting Diptera develop in terrestrial microhabitats (Killick-Kendrick 1999) and our knowledge about their breeding habitats is very limited (Feliciangeli 2004). Therefore, there is an urgent need for the development of targeted, yet environmentally sound, control measures that take advantage of potential weak links in the life cycle of the phlebotomine vector (Warburg and Faiman 2011). The behavior of sugar seeking is one such potential weak link for many hematophagous Diptera, including sand flies (Stone and Foster 2013).

Sand flies are small, nocturnally active insects. Both sexes frequently seek sugar for energy which is obtained from a variety of sources, including nectar, honeydew, and the vascular tissues of plants (Stone and Foster 2013). Only the females, however, seek a blood meal that they use for egg production. It can be argued that sand flies are predominately phytophagous insects, as the majority of adult feeding (all males, and all females, except for an occasional blood meal) is on plant sources.

Sources for obtaining sugars are important determinants of sand fly ecology and sand fly-*Leishmania* interactions, and hence the transmission of leishmaniasis (Schlein and Jacobson 1999). Minimal available energy reserves (≤ 0.55 cal per insect) were found in wild-caught *Lutzomyia longipalpis* (Lutz and Neiva) from Colombia and *Phlebotomus papatasi* (Scopoli) from Iran, suggesting that females of these species probably feed repeatedly on sugar sources in nature to obtain sufficient nutrients for survival and dispersal (Magnarelli and Modi 1988). Abundance, distribution, and activity patterns of sand fly species are strongly affected by the availability of specific sugar sources (Yuval and Schlein 1986, Schlein and Yuval 1987, Schlein and Jacobson 1999, Junnila et al. 2011, Muller et al. 2011).

Lutzomyia trapidoi (Fairchild and Hertig) prefers the same sugars that are major constituents of nectar, leading Chaniotis

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14. ABSTRACT
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(1974) to suggest plant nectar as a sugar source for phlebotomine sand flies. In a series of well-designed experiments, Yuval and his co-worker demonstrated the major component of sand fly nocturnal activity is related to sugar feeding (Yuval and Schlein 1986, Yuval et al. 1988). Similarly, it was shown that the majority of sand flies, of both sexes, were found next to flowering plants (Muller et al. 2011, Yuval and Schlein 1986). Sugar feeding by sand flies from either aphid honeydew or a plant source is highly selective. In laboratory trials with *L. longipalpis*, honeydew derived from *Aphis craccivora* was more attractive than six other honeydews (Petts et al. 1997). In cage studies with wild-caught, starved *P. ariasi*, 65% of the flies fed on honeydew of oak infested with the aphid, and 20% fed on honeydew from unidentified aphids on French bean leaves. However, experiments were negative with three other aphid species (Killick-Kendrick and Killick-Kendrick 1987). In a field study, researchers tested 24 commercially available fruits for their attractiveness to sand flies, and found that the top three attractive fruits were nectarine, cactus fruit, and guava (Junnila et al. 2011). In replicated laboratory studies comparing 20% sucrose solution, fresh honeydew, and nectar from *Hoya* sp, scientists found that significantly more *L. longipalpis* fed on nectar than the sucrose solution or honeydew(Petts et al. 1997). Feeding times were longest on the nectar compared with sucrose solution and honeydew. Flies fed on nectar lived longer than flies fed on sucrose solution, and flies maintained on nectar produced significantly more eggs than those maintained on sucrose solution.

These studies indicate that it may be advantageous to manipulate sand flies' obligatory sugar feeding behavior as a possible way to attract sand flies for surveillance and/or control. Optimized floral odor blends (including chemical mimics) are an essential component of semiochemical traps or baits developed for area-wide pest management of phytophagous species from several orders of insects (e.g., Diptera: Tephritids, Drosophilids; Coleoptera: Chrysomelids, Scolytids; Lepidoptera: Noctuids) (Witzgall et al. 2010).

Research on plant-based attractants is conventionally based around identification of the volatile chemical blends from specific plant materials previously established as attractive to the target insect. While some work suggests the importance of nectar feeding (Chaniotis 1974, Petts et al. 1997, Muller et al. 2011), direct field observations of sand fly floral feeding are lacking, probably due to a combination of nocturnal behavior and the small size of these insects. An alternate approach is suggested based on innovative research methods used to successfully develop baits for Heliothine Lepidoptera using high-throughput olfactory screening and then combining compounds from several plants to produce a supernatural floral blend not found in nature (Del Socorro et al. 2010 a, b, Gregg et al. 2010).

In this study, we designed and used a 3-chamber in-line -olfactometer. The in-line design was selected because it has a greater ability to differentiate between attraction, non-stimulation, inhibition, and repellency (Dogan and Rossignol 1999). This olfactometer is a modification of the modular high-throughput screening system of Grieco et al. (2005) used to evaluate behavioral responses of mosquitoes to chemicals. The dimensions of the olfactometer were the same as the Grieco modules, but they were built with tighter fittings and O-rings to prevent the escape of much smaller sand flies. The internal drums and nets were eliminated and replaced with a tray to hold the test substances. Directional airflow was also incorporated into the device. After passing through a filter, the air was split before entering into two floating ball regulators to regulate and equalize airflow before entering the outer chambers and exiting through a port in the middle of the center chamber.

This research project investigated sand fly orientation to volatiles from diverse honeys, used as a proxy for plant nectar. Unifloral honeys have diverse volatile and non-volatile chemical compositions that represent the diversity of source plant (Cuevas-Glory et al. 2007, Kaskoniene and Venskutonis 2010, Manyi-Loh et al. 2011). We developed and tested a 3-chamber in-line olfactometer as a high-throughput system for screening potential attractants, including the honeys from various sources. The attractive honeys identified in this research could be used in further chemical and behavioral studies towards identifying and optimizing attractive synthetic blends for incorporation into targeted bait technology for area-wide leishmaniasis vector control.

MATERIALS AND METHODS

Monofloral honey

A total of 25 honey samples collected from a wide range of plants assembled from three different continents were screened. Of these, 23 were of a unifloral (nectar predominantly from one plant species) origin and two were an unknown mixture from Rocky Mountain meadows (compound K) and a Western Europe forest (compound H1). Of these, nine were identified to the genus level and 14 to the species level of plants (Table 1). The diversity and uniqueness of volatile blend compositions have been demonstrated for a diverse range of unifloral honeys (Cuevas-Glory et al. 2007, Kaskoniene and Venskutonis 2010, Manyi-Loh et al. 2011) and supports their use in development of putative attractant mixtures as proxy nectar in this research.

Sand flies

We used three sand fly species: two Old World species (*P. papatasi* and *P. duboscqi*) and one New World species (*Lutzomyia longipalpis*). For *P. papatasi,* we used sand flies originating from four distinct geographical locations: Abkuk Turkey (hereafter, PPTK), Israel (hereafter, PPIS), Jordan (hereafter, PPJD), and North Sinai (hereafter, PPNS). For *P. duboscqi* (Neveu-Lemaire), we used sand flies originating from Kenya (hereafter, PDKY) and Senegal (hereafter, PDSN), while for *L. longipalpis,* we used the Brazilian Jacobina strain (hereafter, LLJB) (Table 2). For PPTK and for LLJB, we also compared the attractance of a selected number of honeys between males and females (Table 2). All sand flies used in these experiments were from colonies maintained at the Walter Reed Army Institute of Research using procedures described by Lawer et al. (1991) and Modi and Rowton (1999) which include a reverse light/dark cycle. Sand flies (blood unfed) were tested two to six d after eclosion and were not allowed access to sugar for 12-18 h before testing. Due to variability in the productivity of the different lab colonies, not all sand fly species were screened against all honey sources. PDKY was the species most thoroughly studied (23 compounds), followed by PPTK (11 compounds), LLJB (ten) compounds each), PPIS (eight compounds), PPJO and PDSN (seven compounds, each), and PPNS (six compounds) (Table 2).

Table 1. List of honeys used in the study.

Honey code	Plant name (common name)	Producer / Retail Store	Country of Origin
A	*Epilobium angustifolium* (Mountain Fireweed)	GloryBee Honey	USA
B	*Rubus armeniacus* (Oregon Blackberry)	GloryBee Honey	USA
C	*Centaurea spp*. (Starthistle)	GloryBee Honey	USA
D	*Rubus idaeus* (Red Raspberry)	GloryBee Honey	USA
E	*Fagopyrum esculentum* (Buckwheat)	GloryBee Honey	USA
F	Unknown "Mountain Wildflower"	GloryBee Honey	USA
G	*Trifolium* spp. (Clover)	GloryBee Honey	USA
H	*Salvia officinalis* (Garden Sage)	Rita Millers	USA
J	*Citrus sinensis* (Orange)	Rita Millers	USA
K	*Chamerion angustifolium* (Rosebay Willowherb)	Purple Gold Apiaries	BC, Canada
M	*Eucalyptus* spp.	Rita Millers	USA
O	*Carduus* spp. (Thistle)	Purple Gold Apiaries	BC, Canada
P	*Eucalyptus camaldulensis* (Red Gum)	Woolworths	Australia
Q	*Eucalyptus globulus* (Blue Gum)	Woolworths	Australia
R	*Metrosideros excelsa* (Pohutukawa)	Comvita	New Zealand
S	*Fagus* spp. honeydew (Beechwood Honeydew)	Comvita	New Zealand
T	*Eucalyptus* spp. (Iron Bark)	Capilano	Australia
U	*Eucalyptus melliodora* (Yellow Box)	Woolworths	Australia
V	*Thymus vulgaris* (Thyme)	Adony	Greece
W	*Solidago* spp. (Goldenrod)	Adony	Ontario, Canada
Y	Citrus sinensis (Orange)	Adony	Spain
Z	*Trifolium* spp. (Clover)	Adony	Alberta, Canada
F1	*Echinacea purpurea* (Echinacea)	GloryBee Honey	USA
G1	*Acacia* spp.	Breitskamer, Wild Oats	Germany
H1	Unknown ("Forest Honey")	Breitskamer, Wild Oats	Germany

Table 2. Sand fly species used in the study, their origin, and the monofloral honey tested. The floral identity of honey codes is described in Table 2.

Species	Origin	Acronym	Years in Colony	Sex	Honeys tested
Phlebotomus papatasi	Turkey	PPTK	7	F,M	Female: A, B, C, E, F, G, P, U, Y, F1,G1 Male: A, E, F, F1, G1
Phlebotomus papatasi	Israel	PPIS	>30	F	Female: A, B, C, D, F, P, T, G1
Phlebotomus papatasi	Jordan	PPJO	10	F	Female: A, C, F, G, P, T, G1
Phlebotomus papatasi	N. Sinai	PPNS	24	F	Female: A, C, F, G, P, T
Phlebotomus duboscqi	Kenya	PDKY	28	F	Female: A, B, C, D, E, F, G, H, J, M, O, P, Q, R, S, T, U, V, W, Y, Z, G1, H1, K1
Phlebotomus duboscqi	Senegal	PDSN	~30	F	Female: A, C, F, G, P, T, G1
Lutzomyia longipalpis	Jacobina, Brazil	LLJB	37	F, M	Female: A, B, C, E, F, G, J, P, T, G1 Male: A, C, , E, F, H, J, P, F1, G1

Olfactometer for bioassay experiments

We used a 3-chamber in-line olfactometer (Figure 1), designed and developed by ER for containment and use with small flying insects. The olfactometer was built at the Medical Prototype Development Lab, U.S. Army Medical Materiel Development Activity, Fort Detrick, MD. The olfactometer (Figure 1) consists of three cylindrical Plexiglas™ tubes (each 16 cm in length with a 9 cm inside diameter) aligned horizontally to form three chambers. The chambers are partitioned and held together by Teflon bulkheads with O-rings at the interface. Each bulkhead has a built-in butterfly door. The end caps are also Teflon with a port in the center for air entry. A perforated stainless steel bridge is placed horizontally at the distal end of each outside chamber to hold weigh boats containing test samples. Air is pulled through the system by a vacuum applied at a port in the middle of the center chamber. After being pulled through a filter (Donaldson Ultrafilter® PN 1C22116), the air is split and flows to two floating ball flow meters which are used to adjust the flow into each distal chamber to 1.5 liters/m.

Bioassays were conducted during morning hours (night, considering the reverse light/dark rearing conditions), in complete dark in an environmental room within an AirScience® Portable chemical fume hood room at 26° C and 80% RH. To assay a honey, 0.05 g was added to a weigh boat and placed on the bridge in one of the distal chambers with no treatment control on the other side. A mechanical aspirator was used to transfer 20 sand flies of a specific strain, sex, age, and stage from their holding cage and introduce them through a port into the center chamber of the olfactometer. A hose was attached to the port and a vacuum was used to pull through the system beginning 1 min before the perforated butterfly doors on each bulkhead were simultaneously opened. The doors were left open for 15 min to allow sand flies free movement between the three chambers before the doors were closed and the sand flies in each chamber counted. The assay was replicated at least six times for each strain and sex combination, interchanging the location of the test compound between the distal chambers.

Data analysis

Tests of significant differences between the number of sand flies in the test and control chambers were done using Wilcoxon signed-rank test (with continuity correction), which is a non-parametric equivalent of the paired t-test. Given the *a priori* hypothesis of monofloral honeys being attractants, we used one-sided P-values. A biologically meaningful response was inferred if the statistical effect was either marginally significant ($0.05 \geq P < 0.1$) or statistically significant ($P < 0.05$). Analyses were conducted using R software.

RESULTS

Comparison among geographic populations of the same species

Phlebotomus papatasi. Females of PPIS were the most diverse in terms of their preferences, with attraction exhibited for four honey types (Red raspberry, "Mountain Wildflower", Acacia spp., and Iron Bark) (Figure 2). PPTK and PPJN exhibited preference for two honey types ("Mountain Wildflower," Acacia spp., and Mountain Fireweed and "Mountain Wildflower," respectively) (Figure 2). Among the honey types tested, PPNS was attracted only to Iron Bark (Figure 2). In terms of similarity of preference among the geographic strains, Acacia spp. honey was attractive to both PPIS and PPTK (Table 3). Mountain Fireweed was attractive to both PPTK and PPJN. Iron Bark was attractive to both PPIS and PPNS. Finally, the generic "Mountain Wildflower" was attractive to both PPIS and PPJN (Table 3). Overall, the *P. papatasi* response of either strain or gender to honeys presented was not very strong, with more than 60% of sand flies not making a choice and staying in the central chamber (Figure 2).

Phlebotomus duboscqi. For PDKY females, attraction was demonstrated towards the following monofloral honeys: clover, red gum, iron bark, and a marginally significant attraction to Rosebay Willowherb (Figure 3). For the Senegalese population (PDSN) female attraction was demonstrated towards Mountain Fireweed and Starthistle (Figure 2). No common attractant for both PDKY and PDSN populations was found (Table 3). *P. duboscqi* response of either strain to honeys presented was also not very strong, with more than 50% (most cases except for compound C) of sand flies not making a choice and staying in the central chamber (Figure 3).

Table 3. Honey preference similarity matrix. Honey codes on the main diagonal indicate the strain specific honey preferences. Values below the main diagonal indicate the shared honey preference of different sand fly strain pairs. For honey code, refer to Table 1. For sand fly species code, refer to Table 2.

	PPTK F	PPTK M	PPIS F	PPJN F	PPNS F	PDKY	PDSN	LLJB F	LLJB M
PPTK F	A, G1								
PPTK M									
PPIS F	G1		D, F, T, G1						
PPJN F	A		F	A, F					
PPNS F			T		T				
PDKY F			T		T	G, K, P, T			
PDSN F	A			A			A, C		
LLJB F			F	F				F, J	
LLJB M			F	F		G		F	F, G

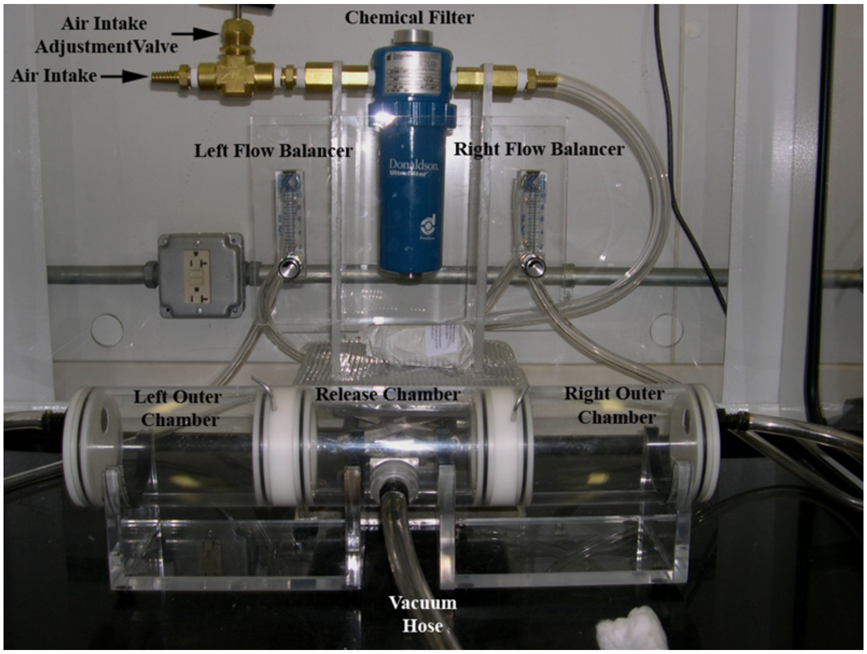

Figure 1. The 3-chamber in-line oflactometer showing the route of filter air through the system. Air enters at the air intake, which has an adjustment valve. After passing through the filter it goes to two flow balancers that regulate the amount of air going into each of the outer chambers. The air is pulled through the system by a vacuum connected to a port in the center of the release chamber.

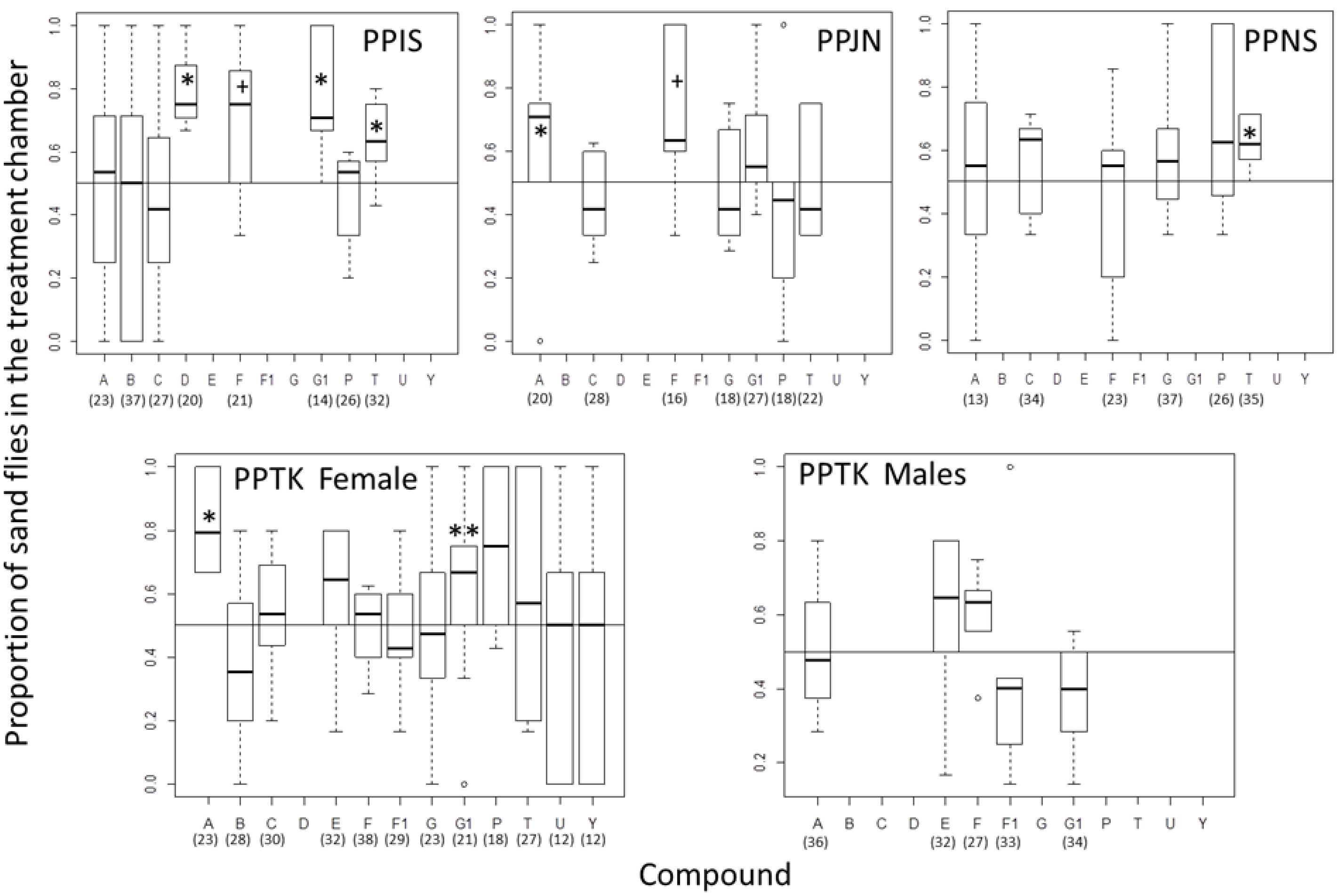

Figure 2. Box plot of the proportion of sand flies found in the treatment chamber of the linear olfactometer for different geographic *Phlebotomus papatasi* strains (PPIS = Israeli strains, PPJN = Jordan strain, PPNS = North Sinai strain, PPTK = Turkey strain) in response to different monofloral honeys. Numbers in parentheses underneath the letter codes of the compounds indicate the mean percent of sand flies making a choice (i.e., not staying in the central chamber). Statistical significance markers (Wilcoxon signed-rank test): ** P<0.01, *P<0.05, +<0.1. For compound key refer to Table 1.

Lutzomyia longipalpis. For both female and male LLJB sand flies, attraction was demonstrated towards "Mountain Wildflower." Marginally significant preference was shown for orange and clover honeys for female and male LLJB sand flies, respectively (Figure 4). *L. longipalpis* response of either gender to honeys presented was not very strong, with at least 55% (most cases except for compound C) of sand flies not making a choice and staying in the central chamber (Figure 4).

Comparison among species

Overall, *P. papatasi* sand flies demonstrated attraction to five honey types (A, G1, D, F, and T) (Table 1) with some overlap between the geographical isolates (Table 3). *Phlebotomus duboscqi* sand flies demonstrated attraction to six honey types (A, C, G, K, P, and T) (Table 1) although the preferences of the two geographic isolates are distinctive (Table 3). For both *P. duboscqi* isolates, some overlap in honey preference exists with *P. papatasi* as well as with LLJB (Table 3). For *L. longipalpis,* attraction to three honey types was demonstrated (F, J, and G) (Table 1). We expected that given their geographic, taxonomic, and ecologic distance, LLJB sand flies would have a distinctive set of honey-type preferences. Surprisingly, with the exception of Orange honey, none of the other honey preferences are distinctive with Clover overlapping with PDKY and "Mountain Wildflower" overlapping with PPIS and PPJN.

Comparison among sexes

We expected sexes to share feeding preferences. For PPTK females, Acacia spp. and Mountain Fireweed were attractive. In contrast, for PPTK males none of the five honeys tested (Table 2) showed a significant or marginally significant attraction (Figure 2). Interestingly, the trends for Mountain Fireweed appeared inconsistent and the trends for *Acacia spp.* even appeared opposite between the sexes (Figure 2). On the other hand, for LLJB seven honey sources were tested for both genders. For LLJB, the multi-floral honey "Mountain Wildflower" brand was attractive to both sexes (although only marginally significant for the female) (Figure 3). On the other hand, Orange honey was attractive only to females, while Clover was attractive only to males (both marginally significant) (Figure 4).

Comparison among honey types

Of the 25 honeys tested, ten had a significant attraction for one or more of the sand fly isolates tested. None of the honeys tested had significant attraction for all the species and geographic isolates tested (Table 3). Mountain Fireweed (PPTK, PPJN, PDSN), Iron Bark (PPIS, PPNS, PDKY), and the "Mountain Wildflower" (PPIS, PPJN, LLJB) were attractive to three sand fly isolates. Acacia spp. (PPTK, PPIS) and Clover (PDKY, LLJB males) were attractive to two sand fly isolates. One the other hand, Red Raspberry (PPIS), Rosebay Willowherb (PDKY), Red Gum (PDKY), Starthistle (PDSN), and Orange (LLJB females) were attractive to only a single sand fly isolate (Table 3).

DISCUSSION

Sugar is the main source of energy for the daily activities of sand flies (Yuval and Schlein 1986, Yuval et al. 1988, Petts et al. 1997, Killick-Kendrick 1999, Schlein and Jacobson 1999, Muller et al. 2011). The major sugar sources for sand flies were traditionally thought to be aphid honeydews (Killick-Kendrick and Killick-Kendrick 1987, Moore et al. 1987, Macvicker et al. 1990, Killick-Kendrick 1999), and directly derived from selected native plants (Schlein and Yuval 1987, Schlein and Muller 1995). More recently, it was demonstrated that sand flies also feed on flower nectars (Petts et al. 1997, Muller et al. 2011) and even fruits (Junnila et al. 2011). However, our understanding of sand fly sugar-feeding preferences remains very limited. The only study to date that has evaluated sand fly nectar preferences is that of Muller et al. (2011) who field-tested the preference of *P. papatasi* to 56 types of flowering plants in an oasis in southern Israel. The top three attractive flowering plants in this study were *Ochradenus baccatus*, *Prosopis farcta*, and *Tamirix nilotica,* all of which are native plants typical to that habitat. Yet, a comparative study of the nectar preference among different sand fly species or geographical isolates necessitates a controlled lab study. Given that honey is produced from nectar, we used monofloral honeys from a variety of old- and new-world commercial sources as a proxy for assessment of nectar preference. Identification of species-specific, sugar-source phytochemical attractants could prove useful for the development of attractive taxa-specific lures that could be used for the surveillance of sand fly species that function as pathogen vectors and, possibly, for the development of an attract-and-kill trap (Schlein and Muller 2010).

We used a 3-chamber in-line olfactometer as a high-throughput method to screen honeys for attractiveness to lab-reared *P. papatasi* (four geographic isolates), *P. duboscqi* (two geographic isolates), and *L. longipalpis.* We were able to detect a biologically-meaningful response to some of the honeys tested. Of the 25 honeys tested, ten had a significant attraction for one or more of the sand fly isolates tested. None of the honeys tested had significant attraction for all the species and geographic isolates tested. At most, three honeys were attractive to three sand fly strains (Table 3). The honeys that had the greatest diversity of attraction were Mountain Fireweed, Iron Bark, and "Mountain Wildflower." None of these honeys came from the native range of these species, so direct coevolutionary adaptation to these plants cannot provide a reasonable explanation for this preference. It is more likely that some component(s) in these honeys exists in some local sugar source (e.g., honey, nectar, honeydew, fruits) in their native habitat. Interestingly, *Acacia* spp. honey was attractive to sand flies originating from Turkey and Israel where Acacia trees are common, in which case co-adaptation could provide a reasonable explanation. Overall, the attraction of the sand flies to different honeys appears to be species- and even strain-specific. Among the four strains of *P. papatasi* tested, each geographical strain had a unique set of attractive honeys with some overlap among these preferences (Table 3). For *P. duboscqi,* honey-type preferences of the Kenyan and the Senegalese populations were distinct. This could possibly be explained by their substantial geographical separation (east- vs west-Africa, respectively) together with the difference in their native biome characteristics (semi-arid vs tropical, respectively), which would

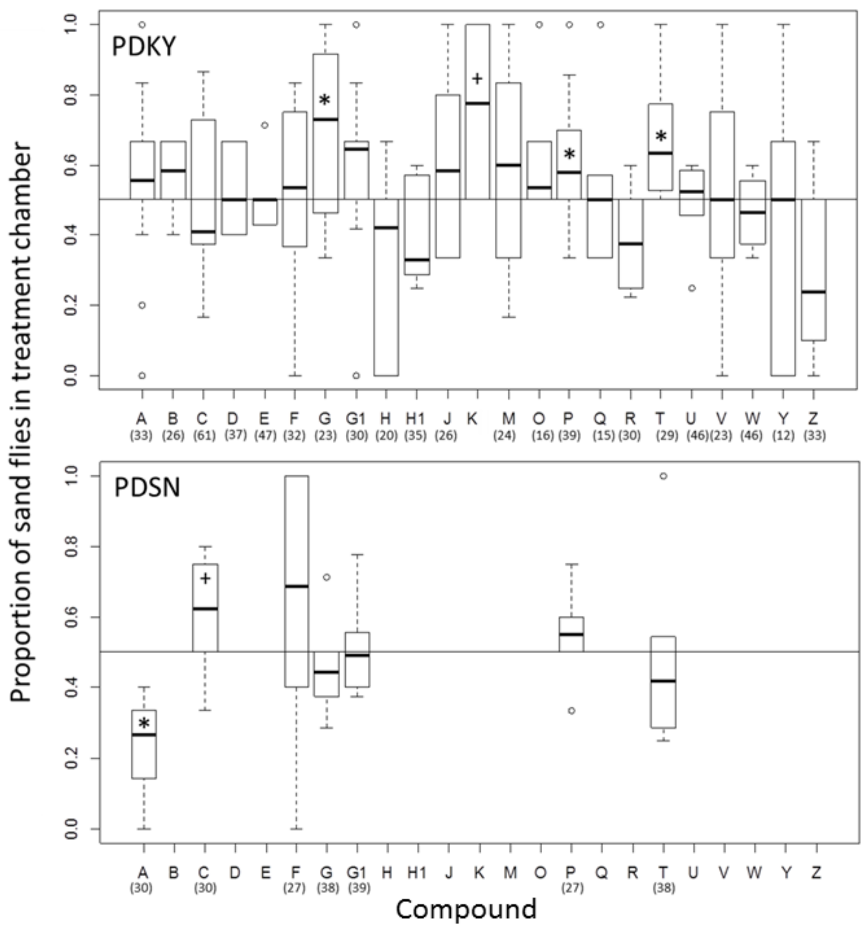

Figure 3. Box plot of the proportion of sand flies found in the treatment chamber of the linear olfactometer for two geographic *Phlebotomus dubosqui* strains in response to different monofloral honeys. Numbers in parentheses underneath the letter codes of the compounds indicate the mean percent of sand flies making a choice (i.e., not staying in the central chamber). Statistical significance markers (Wilcoxon signed-rank test): ** P<0.01, *P<0.05, +<0.1. For compound key, refer to Table 1.

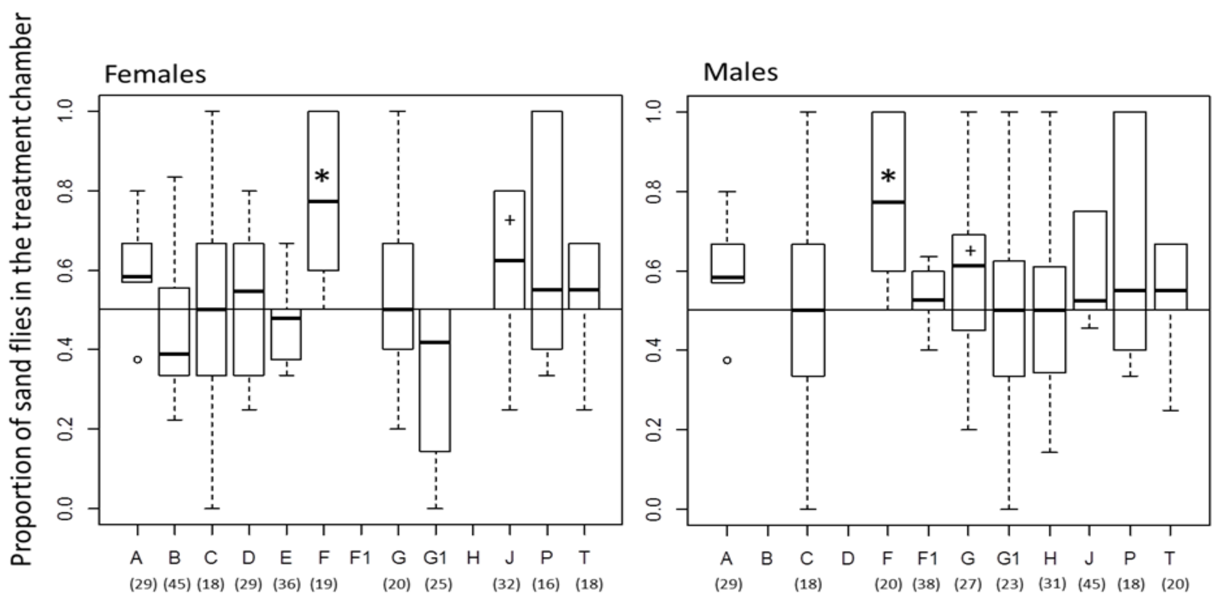

Figure 4. Box plot of the proportion of sand flies found in the treatment chamber of the linear olfactometer for females and males *Lutzomyia longipalpis* in response to different monofloral honeys. Numbers in parentheses underneath the letter codes of the compounds indicate the mean percent of sand flies making a choice (i.e., not staying in the central chamber). Statistical significance markers (Wilcoxon signed-rank test): ** P<0.01, *P<0.05, +<0.1. For compound key, refer to Table 1.

lead to evolutionary differentiation in sugar-source preferences. It was somewhat surprising to find the New World *L. longipalpis* sharing preference for Clover and “Mountain Wildflower” with its Old World counterparts. Yet, attraction for Orange flower honey was distinct to *L. longipalpis*. Finally, we expected sexes of the same species to share preferences for the same honey type. This was shown, for example, by Junnila et al. (2011) who found both sexes of *P. papatasi* to be attracted to nectarines, cactus fruit, and guava in the field. For *L. longipalpis*, both sexes shared their primary preference for “Mountain Wildflower” but differed in their secondary preference, with females preferring orange-based honey and males preferring clover-based honey (Figure 3). In contrast, for PPTK none of the honeys that were found attractive to females were attractive to males. However, this might simply reflect the fact that only a narrow range was tested for males (five vs 12 honey types tested for males and females, respectively) and probably broader screening would detect shared attractive honeys.

Clearly, this line of research should be expanded to obtain a more comprehensive database regarding the response of different sand fly species and strains to the same set of honey types. Furthermore, a more hypothesis-driven approach should be used to evaluate the affinity of sand fly species and geographic strains to native vs foreign plants. The subsequent step would be to use fractionation methods (Ponnusamy et al. 2008) to identify and isolate the specific attractive compounds that drive this behavior and test sand fly responses to these compounds using large-cage lab experiments to be followed by field experiments using blends of the most attractive semiochemicals. Although sand fly response in our study appeared to be species/strain specific, the effect on other non-target insect species of economic or conservation value has yet to be determined. Nonetheless, this line of investigation, addressing sugar feeding preferences of sand flies, holds great promise for the development of an effective targeted sand fly control measure, which as a part of a broader integrated pest management program could reduce the burden of sand fly-borne diseases around the world.

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